# Metabolomics in Pakistan: A Comprehensive Analysis of Availability, Practices, and Challenges in Clinical Laboratories

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## ABSTRACT

**Objective:** To comprehensively analyse the current state of metabolomics in clinical laboratories across Pakistan.
**Study Design:** Cross-sectional survey.
**Place and Duration of the Study:** Section of Chemical Pathology, Department of Pathology and Laboratory Medicine, The Aga Khan University, Karachi, Pakistan, from June to November 2022.
**Methodology:** This survey assessed metabolomics availability, computational methods, and resources in Pakistani clinical laboratories. The survey questionnaire was distributed within the chemical pathology community *via* Google Forms, and responses were garnered from 44 chemical pathologists across 17 laboratories in 10 cities.
**Results:** Among respondents, 11 had formal metabolomics training, with three having less than 1 year, five more than 1 year, and 33 lacking any experience. Primary clinical applications included inherited metabolic disorders, bio-informatics, microbiology, therapeutic drug monitoring, toxicology, and nutritional and environmental science. Thirty-three labs reported no omics-related technology; LC-MS and GC-MS were present in eight labs and capillary spectroscopy, direct infusion mass spectroscopy, and Fourier transform infrared-spectroscopy were predominantly used for metabolite identification. Challenges identified in implementing metabolomics included lack of awareness, limited expertise, low volume, restricted applicability, high instrument costs, and insufficient funding.
**Conclusion:** Metabolomics in Pakistan faces limitations, but the chemical pathology community expresses strong interest, emphasising the need for intensified efforts in education and training in this emerging field.

**Key Words:** *Metabolomics, Chemical pathology, Pakistan, Clinical laboratories, Genetics, Survey.*

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## INTRODUCTION

Metabolomics, one of the most recent 'omics' sciences following genomics, proteomics, and transcriptomics, is a growing field that delineates or maps fingerprints of the 'metabolome' by combining advanced analytical chemistry techniques with sophisticated statistical methods.[1] In the context of metabolomics, which entails the study of metabolites or small molecules produced by the metabolic processes within a living system, the comprehensive analysis of these molecules offers insights into the metabolic pathways and biochemical processes underlying physiological and pathological conditions.[2]

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Metabolomics combines high-throughput analytical techniques with bio-informatics, offering a valuable approach for diagnosing complex disorders, such as inherited metabolic disorders (IMDs), and analysing complex metabolites, including medicines.[3] Metabolomics experimentation strategies are classified as 'targeted' or 'non-targeted'. Targeted approaches involve multiplexed analysis of known metabolites and have proven useful in disease pathology and diagnosis, particularly when disease-specific metabolites are known. Non-targeted approaches, on the other hand, aim to detect unknown or rare metabolites and identify biomarkers that differentiate cases from control groups.[4]

Nuclear magnetic resonance (NMR) and mass spectrometry (MS) are the two primary analytical techniques employed in metabolomics. Both of these techniques can identify and quantify metabolites while analysing a large number of metabolites coexisting simultaneously in complex samples such as blood, urine, tissue, and saliva.[3,5] MS-based metabolomic technology is extensively utilised in clinical research, disease treatment, and drug characterisation, as well as in animal and plant research, agricultural research, and nutrition.[6]

The applications of metabolomics are vast, but the effective utilisation of this technology in diagnostic practice depends on laboratory personnel's understanding of its applications. This field holds the potential to furnish clinicians with new biomarkers for early disease diagnosis, surveillance, and monitoring treatment responses, among other benefits.[7] Metabolomics remains a developing field in many low- to middle-income countries, with ongoing efforts to integrate it into routine diagnostic practice. The purpose of this survey was to evaluate the knowledge and application of metabolomics within the chemical pathology community.

## METHODOLOGY

A cross-sectional survey study design was conducted after receiving approval from the Institution's Ethical Review Committee (ERD ID: 2022-7146-20711). The study was conducted at the Biochemical Genetics Laboratory, Department of Pathology and Laboratory Medicine, The Aga Khan University, from June to November 2022. Residents and chemical pathologists across Pakistan were invited to participate in the online survey. Only those consenting to participate were included while those who were not chemical pathologists or trainees of chemical pathology were excluded. Participants were reached out *via* email and social media platforms, such as WhatsApp groups of chemical pathology. Additionally, the snowball sampling method was utilised, wherein participants were encouraged to share the questionnaire with their fellow chemical pathologists in their respective institutes.

The questionnaire was developed following a literature review as no validated questionnaire was available for the target population. The survey form was created using Google Forms for easy accessibility to the participants. While the form was primarily in English, forms in Urdu were also made available for the participants. The form underwent review and pilot testing by all team members to ensure clarity and comprehensibility of the items.

Comprising three sections, the questionnaire included a total of 24 questions. Questions were evaluated on a three-point scale (correct, incorrect, or partially correct), with a 'do not know' option included to mitigate guessing. The first section assessed healthcare professionals' knowledge of basic metabolomics and its applications. The second section focused on participants' clinical experience and practices in the field of metabolomics, while the final section addressed the need for metabolomics in Pakistan, emphasising training requirements and challenges. Data analysis was conducted using SPSS 23, with results presented as mean and standard deviation (SD) or as frequencies and percentages, as appropriate.

## RESULTS

A total of 44 chemical pathology experts and trainees from Pakistan participated in the survey. Of the total, 54.5% (n = 24) were consultant chemical pathologists, 36.4% (n = 16) were postgraduate trainees, and +9.1% (n = 4) were clinical laboratory technologists. Participants were from six different cities, 36.4% (n = 16) of the participants were from Karachi, 27.3% (n = 12) belonged to Rahim Yar Khan, while in remaining 9.1% (n = 4) each were from Islamabad, Lahore, Swat, and Bahawalpur. Eighty-four percent (n = 37) had received no training and only 15.9% (n = 7) had been formally trained in utilising techniques related to metabolomics. Regarding any other informal training received, 34% (n = 15) of the participants had taken short training courses, 18% (n = 8) had taken online courses, and one participant 2% (n = 1) had taken the long courses and did an attachment at a biochemical genetics laboratory within Pakistan during his residency.

Seven multiple-choice questions (MCQs) were asked to assess the participants' knowledge of metabolomics. The MCQs related to the molecules measured were answered correctly by most of the participants while none responded completely correct for the techniques utilised in metabolomics. The complete questions and the participants' responses are shown in Table I.

The second section focused on the questions centred on the participants' experience with metabolomics, bio-informatics, and metabolomics-related technology, as well as the challenges they faced when introducing metabolomics into their respective labs. It had seven questions. The vast majority of participants, nearly 84% (n = 37), had no prior experience with metabolomics, while the remaining 6.8% (n = 3) had less than a year of experience and another 9% (n = 4) had more than one year of experience. On inquiring about the areas they are utilising metabolomics techniques, the majority 45% (n = 20) participants did not employ metabolomics in their work while IMDs received the most responses 45% (n = 20), as shown in Figure 1.

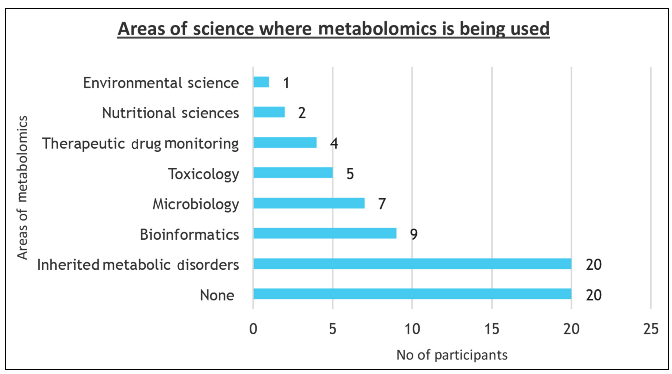


**Figure 1: Application of metabolomics by the participants.**

In regard to the metabolomics-related technology / instruments they had available in their lab, almost 75% (n = 33) said none, while a few had multiple instruments available; 15% (n = 7) were using Liquid Chromatography-MS and Gas Chromatography-MS, 11.3% (n = 5) were using NMR, and 9% (n = 4) were using Capillary Electrophoresis-MS, Direct-Infusion-MS, and Fourier-MS. The participants were then asked about the type of bio-informatics they were currently performing. Almost 73% (n = 32) of the participants answered none, while a few 13.6% (n = 6) responded that they were using metabolite identification followed by biostatistics 2.5% (n = 1) and network analysis 2.2% (n = 1).

**Table I: Knowledge assessment of metabolomics among chemical pathologists and trainees.**

| Questions in the survey | Correct answer | Correct response % (n) | Partially correct % (n) | Incorrect response % (n) | Do not know % (n) |
|---|---|---|---|---|---|
| Metabolomics involves the study of small molecules which include? | Metabolites | 63.6% (38) | - | 34 % (6) | - |
| Metabolomics usually studies molecules within a mass range of: | Less than 1500Da | 59% (26) | - | 13.6% (6) | 27.2% (12) |
| Which of the following classes of molecules could be included in a metabolome? | Sugars, lipids, amino acids, nucleotides, cofactors, drugs, hormones, toxins, and mRNA. | - | 43% (19) | 56.8% (25) | - |
| Sample types commonly studied in metabolomics include: | Saliva, blood, urine, faces, tissue, serum, cerebrospinal, fluid, and hair. | 9% (4) | 39% (17) | 48% (21) | 5% (2) |
| Steps of metabolomic analysis include: | Extraction, derivatisation, molecular modelling, separation, detection, analysis, deproteinisation, and cell lysis. | 2.27% (1) | 29.5% (13) | 61.3% (27) | 6.8%% (3) |
| Commonly used analytical methods in metabolomics: | 2D chromatography, HPLC, gas, chromatography-mass spectrometry, Fourier mass spectrometer determines, MALDI-TOF, nuclear magnetic resonance spectrometry, electrophoresis, and liquid chromatography- mass spectrometry. | - | 56.8% (25) | 40.9% (18) | 2.2% (1) |
| Nuclear magnetic resonance spectroscopy is more sensitive than mass spectrometry. | False | - | 29.5% (13) | 61.3% (27) | 9% (4) |

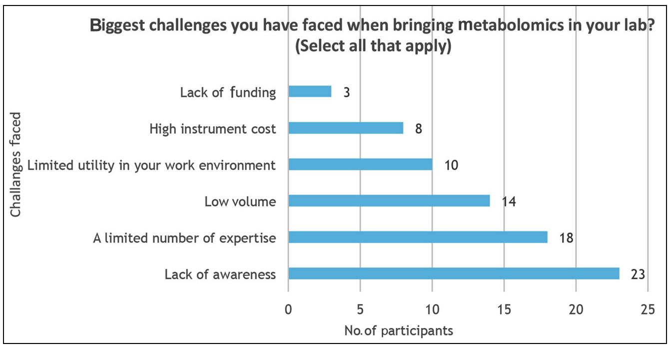


**Figure 2: Challenge encountered by participants when bringing metabolomics to the lab.**

The participants were using open-source for data processing (e.g., peak picking, filtering, and alignment) and data analysis. The most common challenge encountered by the participants when bringing metabolomics to the lab is shown in Figure 2.

The authors then asked the participants if they thought Pakistan need metabolomics, and 90% (n = 41) agreed and only 6.8% (n = 3) said no. In terms of training the most popular biological / chemical laboratory techniques in which participants wanted to be trained were chromatogram interpretation and metabolite identification 72% (n = 32), followed by targeted analysis 70% (n = 31), sample analysis on Gas Chromatography-Mass Spectrometry 59% (n = 26), sample analysis on Gas Chromatography-MS 59% (n = 26), quality assurance of MS-based instruments 50% (n = 22), sample analysis on Liquid Chromatography-MS 48% (n = 21), sample preparation 45% (n = 20), sample analysis on NMR 43% (n = 19), and untargeted analysis 36% (n = 16).

The majority 90% (n = 40) wanted training related to IMDs diagnostics, followed by therapeutic drug monitoring 59% (n = 26), toxicology 45% (n = 20), nutritional sciences 23% (n = 10), environmental sciences 18% (n = 8), and microbiology 16% (n = 7).

When asked about the strategy of education and training, 77% (n = 34) participants responded that hands-on workshops should be conducted, 75% (n = 33) responded that online resources should be shared, 56% (n = 25) responded that CME programmes or seminars should be arranged. Fourteen participants (31%) suggested that a fellowship in metabolomics should be initiated.

## DISCUSSION

Metabolomics, a vital tool in medicine, still faces significant awareness gaps. This study reveals a lack of metabolomics' knowledge among participants, highlighting the urgent need for training and awareness. The results provide insights into the surveyed participants' comprehension of key metabolomics concepts; while respondents understood the basics, there was a notable lack of understanding regarding techniques, instrumentation, and applications. Haque *et al*. reported that lower-middle-income countries face challenges in implementing metabolomics and other precision medicine tools due to a lack of population-specific data, a dearth of experts, and poor financial support.[8]

The study also explored the expertise of the participants and areas where metabolomics techniques were employed. The majority had no prior experience, and most users were utilising these techniques in IMDs diagnostics and toxicology. Similar findings were reported by Odom *et al*., indicating that while metabolomics is most frequently used in the diagnosis of IMDs, it is also employed to aid in the diagnosis of disorders such as autism, cancers, and congenital anomalies.[9] The authors further stated that this technique has the potential to replace traditional analyses as a routine evaluation tool for IMDs.

The main challenges identified in this study were the lack of awareness and expertise. Additionally, a few participants felt that metabolomics is not financially feasible and may have limited utility in their work environment. A study by Winder *et al*. reported that one of the major challenges in delivering metabolomics education and training is the diverse background of the trainees, resulting in variable levels of basic metabolomics knowledge. Consequently, the majority of respondents felt the need to develop curricula for PG education, as it is not currently covered in existing PG curricula.[10]

Participants expressed a strong desire for training in various techniques, particularly those focused on IMDs. The study by Sandlers *et al*. discussed the potential of metabolomics in understanding the complex metabolic perturbations associated with IMDs, highlighting both the challenges and prospects of targeted metabolomic studies for these disorders.[11] In this study, the most popular approach was hands-on workshops, followed by in-person training sessions, while online recorded and live webinars were the least preferred methods. Similar findings were reported by Weber *et al*., which highlighted a face-to-face course spanning over 3 days as the preferred training method.[12]

To advance metabolomics' knowledge among Pakistani chemical pathologists, a multifaceted strategy is needed.[13] Developing a comprehensive curriculum, focusing on the identification of metabolites, chromatogram interpretation, and conducting hands-on workshops is also needed. Literature has shown that biochemical genetics is the area, where this metabolomics is most frequently applied globally, hence metabolomics workshops focusing biochemical genetics can be organised.[14] Online resources, fellowships, and collaborative partnerships with international experts aim to supplement training.[10] Advocating for curriculum integration and raising awareness through campaigns will further ensure the sustainable incorporation of metabolomics into clinical practices, fostering a skilled community equipped to address the evolving challenges in precision medicine.[15]

Limitations of this study include a small sample size of 44 participants, potentially limiting generalisability. Self-reported data and survey design may introduce response bias and limit nuance.

## CONCLUSION

Metabolomics research has come a long way since its inception. Currently, knowledge and applications of metabolomics are limited in the chemical pathology community. Within the community, appropriate training and educational initiatives are required to develop this knowledge and skills. Educational initiatives which are both feasible and effective should be taken.

**ETHICAL APPROVAL:**
The study was conducted after receiving approval from the Institution's Ethical Review Committee (ERD ID: 2022-7146-20711).

**PARTICIPANTS' CONSENT:**
Only those participants who provided consent were included in the study.

**COMPETING INTEREST:**
The authors declared no conflict of interest.

**AUTHORS' CONTRIBUTION:**
HM, AI, LJ, AHK: Study conception and design.
HM, AI: Data collection.
HM: Analysis and interpretation of results.
AI, LJ, AHK, IS, SA: Critical review of the manuscript.
All authors approved the final version of the manuscript to be published.

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